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Confocal Endomicroscopy: Clinical Perspectives

Khek-Yu Ho
Clinical Director, Endoscopy Centre, National University Hospital, Singapore

Abstract

Confocal endomicroscopy is an emerging in-vivo imaging technology currently being assessed for its potential in enhanced endoscopic detection and diagnosis of gastrointestinal diseases, specifically in the screening for pre-malignant lesions. Its forte is in the integration of powerful microscopic technology to conventional videoendoscopy, making it capable of generating high resolution images of optical sections of the gastrointestinal epithelium, as far as 250 μm beneath the surface. By enabling the microscopic assessment of both cellular and subcellular morphology of intact living tissue, it makes real-time histopathologic evaluation possible, facilitating instant diagnosis during ongoing endoscopy so prompt decision on therapeutic intervention could be made. With its submicron-level surface and subsurface imaging capability, confocal endomicroscopy can potentially uncover subtle pre-neoplastic mucosal changes and lesions otherwise imperceptible with conventional wide-field videoendoscopy. Where necessary, it can provide more precise targeting of sites for biopsy, thereby reducing chances of biopsy misses and the number of biopsies that needs to be taken. Thus far, results from prospective clinical studies on the utility of this innovative endoscopic device have been highly optimistic. If proven feasible, its implementation in clinical practices could facilitate virtual diagnosis and potentially accelerate therapeutic interventions and improve patient outcomes. This review presents the fundamental working principles of the novel confocal endomicroscope, discusses its capabilities and prospective niches in gastroenterologic applications, and offers a clinical perspective of its potential role in the screening, surveillance and management of gastrointestinal diseases.

Background

Since the introduction of modern flexible endoscopes with enabling fiberoptic technology some forty years ago, there has been considerable growth in endoscopic applications. Over the years, we have witnessed numerous improvements to the design of the endoscope, with efforts focused on refining the mechanics of existing prototypes to optimize usability and increase sensitivity. An arsenal of accessories has also been added, broadening its capabilities considerably. Today, the fiberoptic endoscopes of yesteryears have been largely replaced by the now commonly used electronic videoendoscopes, the resolution of which has improved steadily over the past few years. The most recent range are equipped with 850 000 pixels density charge-coupled device (CCD) image sensing chips; more advanced ones are fitted with motorized zoom lenses which can further augment its magnifying capacity to as much as 150-fold. With that, visualizing even tiny circumscribed lesions in the gastrointestinal mucosa is no longer a challenge.

Decades in the making, today's endoscope is a diagnostic and therapeutic tool of choice and endoscopy is now part of the standard repertoire of procedures in the initial clinical evaluation of gastrointestinal diseases. However, yet unmet needs such as accurate detection of pre-neoplastic mucosal changes and real-time in-vivo histopathologic analysis have engendered interest in developing even more sophisticated optical imaging devices capable of not just superficial but also in-depth imaging of tissue, and at micrometer resolution. As newer technologies continue to spur innovation, several such advanced imaging systems permitting unprecedented live views of biological structures are emerging; among them are optical coherence tomography, Raman spectroscopy, confocal fluorescence endoscopy, autofluorescence endoscopy, laser-induced fluorescence spectroscopy, chromoscopy, reflectance spectroscopy, light-scattering spectroscopy and trimodal

spectroscopy[1,2] As yet, it is uncertain which of these will supersede the others to become the next tool of choice for regular clinical imaging. Herein, we review what we think is a particularly noteworthy and promising novel innovation-the laser-scanning confocal endomicroscope.

Confocal endomicroscopy: The technology and its capabilities

As the name implies, confocal endomicroscopy is essentially a technologic amalgamation of conventional endoscopy and confocal microscopy (Figure 1)-a well-established imaging technique that makes use of molecular information derived from light-tissue interactions to generate images of the tissue. The confocal endomicroscope is a fully functional videoendoscope integrated with a miniaturized confocal laser microscope at its distal tip. It combines the familiar features of the videoendoscope with the superior optical power of confocal microscopy, providing thus a tool that is useful for macroscopic, as well as microscopic assessment of target biological tissue. At the heart of this novel innovation is the optical fiber that serves to both deliver the excitatory laser beam to illuminate the area in focus and to recapture and transmit back the ensuing emittance.

Endomicroscopy adapts the common light microscopy technique in which low-energetic laser illumination is focused onto a single point in a microscopic field of view. To scan an object, a pinpoint blue laser light (488nm) is delivered via the fiber optic coupler and projected onto a diffraction-limited focus point on the tissue. The fluorescence emitted on excitation is captured by the photodetector-a photomultiplier tube preset to detect light within the spectral bandwidth of 505nm to 585nm - through a pinhole aperture positioned in a conjugated focal plane to the focused point. Emittance captured from successive points are electronically processed and encoded as grey scale values based on which an image of the scanned region can be constructed. Spatial filtering eliminates out-of-focus interferences, so, effectively; only light emanating back from the in-focus plane can reach the detector. By rapidly scanning a laser beam across the object in a raster sweep pattern, a full two-dimensional image of the plane is constructed. Each resultant image represents an optical section of just one focal plane within the specimen. Thus, images of a stack of optical sections can be attained simply by varying the axial position of the focal plane through the depth of the tissue to be scanned. There is however, a fiber-limited lateral (across image) and axial (slice thickness) resolution of 0.7 μm and 7 μm, respectively, and a maximum penetration depth of about 250 μm.

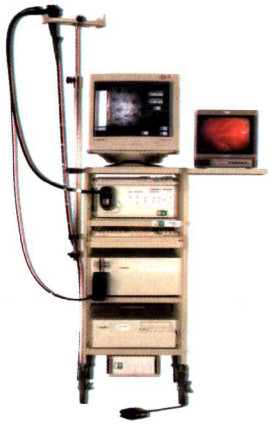

Figure 1 Confocal endomicroscope system

The distinctiveness of this innovative endoscope lies in its capability to generate high resolution (1-2 μm) images of optical sections of intact tissue, hundreds of micrometers beneath the surface epithelium, enabling thus in-vivo microscopic assessment of cellular and subcellular morphology. The incorporated laser scanning confocal microscope is capable of magnifying the structural details up to 1500 times-ten times more than what is achievable by the best conventional endoscopes-rendering microarchitectural cellular details otherwise not evident, or at best, only poorly visualized in conventional videoendoscopy[3]. However, this confocal

endomicroscopic imaging is based on epifluorescence. As such, biomolecules within the tissue to be examined must first be selectively tagged with an exogenous source of fluorophores. For in-vivo use, photosensitizers such as fluorescein sodium, acriflavine and proflavine have been employed to pre-label specific intracellular or intratissular structures before the scanning procedure. On photodynamic activation, the fluorophores taken up by these structures will emit fluorescence, the intensity of which will depend on specific uptake of the fluorophores by different cellular components which varies with tissue properties that change through progressive stages of disease transformation.

Clinical perspectives: Where confocal endomicroscopy might play a potential role

As gastrointestinal cancer remains a leading cause of cancer death worldwide, its clinical diagnosis-most importantly, the early detection of it-is a major concern[4]. Currently, the diagnosis or differential diagnosis of gastrointestinal cancers rests almost entirely on its visual examination and the interpretation thereof, whether in-vivo or ex-vivo. More often than not, more than one of several wide-ranging methodologies encompassing diverse technologies is employed for the detection and evaluation of the disease. Irrespective of the techniques employed, the diagnostic goals remain: (i) soonest detection of the lesion; (ii) determination of the extent of invasion to enable staging; and (iii) ascertaining the differentiation status to grade the malignancy. While detection of the lesion usually involves a thorough scan of a large part of the gastrointestinal lumen, grading of the malignancy requires microscopic observation of morphologic details and staging it necessitates penetration beyond the mucosal surface to variable depth to observe and evaluate spread to deeper tissue structures. Presently, no one single diagnostic modality can accomplish all these alone. While various combinations of existing modalities may be employed, white light videoendoscopy with biopsy remains the mainstay of these investigations. Ex-vivo histopathologic examination of biopsy specimens is the most commonly used technique for prognostic evaluation and is the gold standard for final diagnosis of neoplastic lesions of the gastrointestinal tract, including their grading and staging.

As in any malignancy, detection and intervention at an early stage of pre-neoplastic development is extremely crucial for improving therapeutic outcome and survival of patients. However, the stark reality is that more often than not, lesions that are detected endoscopically are already at a more advanced stage. In the surveillance of high-risk individuals predispose to the development of adenocarcinoma, the detection of dysplasia within the gastrointestinal mucosal layer is a particularly critical threshold stage for intervention to effectively forestall its malignant progression. Unfortunately, inherent shortcomings in conventional wide-field videoendoscopy make it suboptimal for the detection of such early mucosal changes; it also tends to miss more subtle, minute or flat lesions such as flat adenomas. In fact, back to back or repeated colonoscopy showed that up to 27% of adenomas could be so missed[5]. Although newer videoendoscopes (850 000 pixel density) are a lot more sensitive than their predecessors (100 000 to 300 000 pixel density), with the state-of-the-art zoom-enabled magnifying high-resolution endoscopes allowing distinction of mucosal details such as pits and crypt openings, they generally still lack the sensitivity required for detection of visually inconspicuous pre-neoplastic or neoplastic development. Moreover, highly transformed mucosa such as in long-standing chronic ulcerative colitis and in Barrett's esophagus poses additional hindrances in the visual discrimination of dysplasia, even for experienced endoscopists[6]. Up to 45% of ulcerative colitis patients with high-grade dysplasia are reportedly found to have coincidental colorectal cancer that was not evident

endoscopically on initial screening.

Nevertheless, the current clinical challenges in endoscopy are not insurmountable though. For one, the emerging confocal endomicroscopy, with its superior optical capability coupled with the ability to gather in-situ histopathologic information during ongoing endoscopy, offers considerable promise in fulfilling at least some of the currently unmet needs. It is technically well poised to take over those roles that conventional endoscopic technology fails to support or cannot perform as well. From the clinical perspective, a leading specific application for which the confocal endomicroscopy is expected to offer substantial benefit is in the selective endoscopic surveillance of high-risk individuals and patients already presented with dysplasia. For obvious reasons, another almost certain niche role for confocal endomicroscopy is in the screening, detection and surveillance of dysplastic developments in Barrett's esophagus-the most common precursor for adenocarcinoma of the oesophagus and oesophagogastric junction - and also in chronic ulcerative colitis where conventional videoendoscopy has proved to be less sensitive[7]. Less specifically though, if cost does not prohibits, it could provide more effective general screening and diagnosis of gastrointestinal pre-malignancies as it is more capable in detecting subtle alterations to the mucosal surface that often do not show up with white light videoendoscopy and related techniques. In any case, the endomicroscope will offer the opportunity to thoroughly assess cellular morphology in-situ and the ability to acquire in real-time diagnostic information otherwise only attainable through ex-vivo histopathologic examination. Indeed, in more definitive situation, it should help eliminate the need for excisional biopsy altogether, but in general, it is expected to provide better targeting of site for biopsy sampling and improve diagnostic yields.

Clinical Studies: Assessments of confocal endomicroscopy in potential applications

As confocal endomicroscopy is a new diagnostic modality that has been made technologically viable only quite recently, clinical experience on the use of the system is quite limited. The feasibility of confocal endomicroscopy in various potential applications in gastroenterology has been assessed, albeit to a limited extent, in a few pilot and prospective clinical studies conducted in Europe, America and Asia. Results obtained thus far have been quite impressive. All these studies have reported attaining remarkably high-resolution endomicroscopic images of the gastrointestinal mucosa and subsurface area. The quality of the optical cross-sections derived was comparable to those obtained from conventional ex-vivo histopathologic staining technique. Distinct morphologic characteristics of the gastrointestinal epithelium like gastric and colonic crypts and pits, and subsurface cellular and intercellular structures such as gastric glands, epithelial cells and various intercellular features were all readily apparent from the images. Morphologic hallmarks of various gastrointestinal pre-malignant and malignant lesions were also characteristically distinguishable from the normal structures.

In a preliminary assessment of the novel technology, our team evaluated a prototype of the instrument (EC3870; Pentax, Tokyo, Japan) on gastric cancer patients with encouraging results[8]. By correlation with standard ex-vivo histopathology, we were able to establish specific confocal endomicroscopic diagnostic features for normal and diseased gastric mucosa. Using the distinguishing confocal endomicroscopic features for normal gastric mucosa (Figure 2), chronic gastritis, intestinal metaplasia and cancer, we were able to predict the presence of gastric cancer from confocal endomicroscopic images with a degree of accuracy that approached that of histopathologic assessment of the same (accuracy 80%; sensitivity 84%; specificity 95%).

Diagnoses of intestinal metaplasia and gastric carcinoma could be made with reliable inter-observer agreement. We demonstrated that with confocal endomicroscopy, immediate in-vivo diagnosis of gastric mucosal neoplasia and pre-neoplasia is possible.

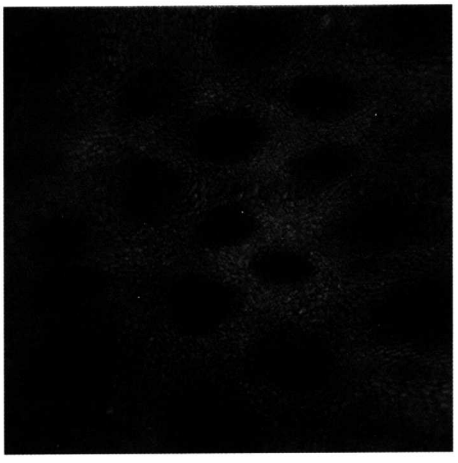

Figure 2 Regular arrangement of the gastric pits.

The technical reliability of the same endomicroscopic system in gastric cancer diagnosis had also been assessed by Kitabatake et al who applied the same technique to gastroscopic differentiation of various normal and diseased gastric mucosa[9]. They demonstrated that the diagnostic differentiation based on confocal endomicroscopic images were as reliable as those obtained from histopathologic examination of the biopsies. The confocal endomicroscopic images they took of the gastric fundic and pyloric mucosa revealed distinctly features characteristic of normal and of metaplastic condition; the presence of gastric cancer was clearly evident from the characteristic disordered configuration of the glands.

Using a different but fundamentally similar prototype (Optiscan, Australia) Burg et al also demonstrated the superior optical capability of the confocal endomicroscope in the determination of gastric cancer[10]. With morphologic examination with histology as reference, they demonstrated that the confocal endomicroscope could generate images with insightful details of tissue morphology, including that of subcellular structures. They reported capturing a clear architecture of colonic crypts and distinctive shapes of epithelial cells. The images also exhibited morphologic details of the perieryptal connective tissue, microvascularization of the stroma and necrotic debris. They thus deduced that the confocal endomicroscope could, apart from assisting in better guidance to the optimal biopsy site, aid in differentiating lesions more precisely.

The superior capability of this technology had also been shown in the detection of colonic dysplasia in a general screening population[11]. Using the same prototype confocal endomicroscope as employed by us[8], Kiesslich et al successfully diagnosed intraepithelial neoplasias and colon cancer during ongoing colonoscopy; they managed to identify neoplastic changes in colorectal mucosa with remarkably high sensitivity (97.4%) and specificity (99.4%), achieving an overall accuracy (99.2%) comparable to that obtained in regular colonscopy plus ex-vivo examination of biopsies. The images they obtained revealed distinctly cellular structures such as crypt arrangements and goblet cells distribution, the examination of which enabled a highly accurate prediction of subsequent histopathologic diagnosis of colon neoplasias in patients. They concluded that confocal laser endomicroscopy is a highly sensitive tool for the screening of flat and polypoid lesions in the colon.

Kiesslich et al further applied this technique to the in vivo diagnosis of Barrett's epithelium and associated neoplasias in patients with gastroesophageal reflux disease with equally remarkable results[12]. Endomicroscopy allowed clear discrimination between different types of epithelial cells and could detect cellular and vascular changes in Barrett's epithelium at high resolution during ongoing endoscopy. They predicted Barrett's esophagus and associated neoplasias with a sensitivity of 98.8%/91.7% and a specificity of 94.4%/99.0%, respectively. The accuracy in the detection of both gastric and

Barrett's epithelium, including Barrett's associated neoplastic changes was at 97.5%. The remarkable results indicated that confocal endomicroscopy might be helpful for surveillance of gastroesophageal reflux patients who are at high-risk of developing Barrett's esophagus that is known to eventually progress to gastrointestinal cancers.

Pitfalls, limitations and future directions

Although confocal microscopy has seen breathtaking advances in the past many years it has been in use and its applications in the identification of cellular and subcellular microstructures within their natural environment have been extensively exploited, techniques and protocols optimized and employed in experimental studies are unfortunately not readily adaptable to in-vivo clinical use in human due to various constraints and safety issues. For obvious reasons, pioneering prototypes of the innovative confocal endomicroscopy such as the ones used in pilot clinical studies reviewed herein are expectedly quite basic when compared to its better developed table-top counterparts and as such, are relatively less versatile functionally. Besides, being an entirely new system, its application in individual situations is still subjected to much needed trial and optimization of pertinent parameters.

Certainly, for those used to the extensive features of table-top confocal microscopy, one feature going to be most missed is the option to render images in three-dimensional volumetric form. Three-dimensional images, though not indispensable, are important for certain functions like in the facilitating of easier differentiation of small pendulous growth such as polyps from complex mucosal folds; in cross sections, both might appear as circumscribed lesions. Current prototypes of the confocal endomicroscope are not built to support true three-dimensional imaging. Although theoretically, a three-dimensional and volume reconstruction of the scanned area is possible, the process of generating such images by stacking contiguous two-dimensional optical sections is cumbersome and difficult in the context of the in-vivo working environment; the main challenge being the maintenance of lateral stability in vertical alignment while optically sectioning through the tissue as all the sections generated must be vertically aligned, failing which the rendering of three-dimensional images may not make sense. Unfortunately, for the existing prototype endomicroscope, acquisition of virtual reality images of the endoluminal view will still have to rely on the parallel imaging of the accompanying videoendoscopic system which is limited in resolution.

The confocal endomicroscope is basically an epifluorescence imaging system for which the quality of the images generated will rely substantially on the optimal use of contrast stains. Unfortunately though, most of the high contrast fluorescent agents available for research purposes are not suitable for in-vivo use, leaving the choices of usable photosensitizers quite limited. In particular, there is a paucity of non-toxic agents that could enhance the visual characteristics of both normal and abnormal tissue and delineate lesions distinctly. Most clinical studies performed to-date have employed topical application of acriflavine hydrochloride or the intravenous administration of fluorescein sodium; both are applicable, but there are limitations: acriflavine hydrochloride cannot penetrate beneath the gastrointestinal epithelium and is therefore ineffective for subsurface imaging, on the other hand, although fluorescein sodium stains adequately to allow clear imaging of morphologic boundaries and distinction of neoplastic tissue, it fails to stain the nuclei of the epithelial cells. Nuclei atypia is an important criterion for the grading of neoplasia. Exploiting of its diagnostic utility would thus have to await development of more selective contrast media, especially one that is highly selective for neoplastic tissue. In our experience, what seems also critical to the image's clarity is the optimum cellular uptake of the

fluorescent stain which is dependent on the duration between administration of the contrast agent and the acquisition of the images. Thus, an immediate challenge to undertake would be the optimizing of staining conditions of existing agents to achieve absolute consistency in the quality of endomicroscopic images acquired. Standard scanning protocol for optimal resolution could then be developed in due course.

Technically however, pilot studies on the utility of the endomicrosopic device in the detection and diagnosis of gastrointestinal diseases did not report encountering any major technical hurdle. The only annoyance felt was minor interferences of upper gastrointestinal tract imaging by cardiorespiratory movements, as reported by Kitabatake et al, who suggested that further refinement in the technology may be required to resolve that. 9 In our own experience though, image fuzziness due to such confounding background artifacts could be attenuated by the stabilizing of the tip of the scope to the gastrointestinal wall with a transparent cap. While application skills can always be honed with more practices, technical pitfalls such as this are best rectified by refining the system. A feasible alternative soft option would be to incorporate a suitable computing algorithm to characterize and cancel noise from all background artifacts. This should not only prove useful in surmounting interferences such as these but also possibly overcome potential interferences from endogenous fluorophores as may be expected in certain conditions such as in extensive inflammation.

What is even more challenging is the present glaring lack of a set of well-defined diagnostic criteria for the interpretation of confocal optical images so derived. Current criteria for distinguishing lesions are very limited and at best, rudimentary; most being largely based on limited confocal features identified and established by individual investigating groups using ex-vivo histopathologic criteria - the current gold standard for the final diagnosis of gastrointestinal diseases - as cross-reference. Hence, it remains for investigators to further define imaging features, compare and achieve a common consensus to standardize these criteria so as to facilitate better interpretation of the images in future. Perhaps, along with this, the system should also be strengthened with the incorporation of sophisticated image analysis software that is able to make computerized histopathologic comparison against standard disease criteria to further facilitate endoscopic diagnoses; such diagnostic criteria integration could enable instant extraction of the most relevant information to facilitate in-vivo diagnosis and expedite clinical decision making.

Conclusion

Although the potential clinical utilities of the confocal endomicroscope is far from being fully investigated and realized, clinical assessments thus far indicate that the confocal endomicroscope, with its superior optical capability to characterize mucosal and subsurface tissue at the micro-structural level, may significantly enhance the ability to diagnose pre-malignant gastrointestinal lesions well beyond that currently achievable by wide-field white light videoendoscopy. Its ability to deliver in real-time much of the diagnostic information otherwise only attainable through ex-vivo histopathologic examination offers a viable alternative to excisional biopsy and the opportunity for endoscopists to draw proper diagnostic and therapeutic decisions during ongoing endoscopy. Confocal endomicroscopy could also potentially reduce the need for biopsies, lower the chances of biopsy misses and improve sampling yields by enabling better visual resolution and more precise targeting of biopsy sites. With it, real-time virtual endoscopic diagnosis is a realistic goal that, when realized, will expedite diagnosis and therapeutic intervention considerably and potentially improve clinical outcomes. However, it must be noted that evaluation of the system in large controlled, randomized clinical trials to fully assess its efficacy and safety is still awaited. Whether it will

eventually be integrated into routine clinical practices for the endoscopic screening, surveillance and management of gastrointestinal disease will depend on the conclusion derived from such evaluation and on future refinement of the technology.

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（本文由*Pentax*公司提供）

内镜超声引导下无水酒精直接注射治疗胰腺癌

杨秀疆 任大宾 刘苏 谢渭芬 沈建伟 蔡洪培 朱焱

胰腺癌诊断时80%病例已属晚期，手术切除率20%左右，术后累计总生存率小于1%。包括手术治疗在内的综合治疗是治疗胰腺癌的主要方法，总体疗效很差。近年来健择(gemcitabine)主导的化疗疗效在10%～20%，但其作用只限于肿瘤稳定而非使瘤体缩小。酒精注射瘤体造成凝固性坏死，直接减少瘤负荷，对很多实体肿瘤均是有效的方法，比如肝癌，其疗效堪与其他任何方法者相比。但在胰腺癌，因顾及并发胰腺炎，只在手术中因无法切除病灶做一次性注射，因准确程度问题及创伤大无法普及，至今尚无人做过不开腹酒精直接注射胰腺癌治疗的报道[1,2]。本文在充分预防胰腺炎的基础上，以超声内镜准确定位及近乎直视下对10例胰腺癌直接注射无水酒精治疗，取得较好疗效，现报道如下。

病例和方法

一、病例

收集我院2004年11月10日～2005年6月在我院住院的10例不能手术的胰腺癌患者，男性8例，女性2例，平均年龄60.53±11.26岁。各例均经实验室检查B超、CT及超声内镜影像学检查及EUS引导下病理确诊。术前综合判定无法手术切除。有两例肿瘤虽小(2.5 cm，3.5 cm)，但年龄超过75岁，心肺功能不佳，仍确定为不能手术对象。

二、方法

1. 术前处理：10例均术前均做ERCP胆管支架置入，其中2例为体尾部胰腺癌因肝脏转移，也行胆管支架置入。术前输注环丙沙星0.2预防感染、甲磺酸加贝酯300 mg预防胰腺炎。腹腔神经丛阻滞止痛者术中以生理盐水维持输注。

2. 器械及药品：本文用Pentax-36UX纵轴超声内镜，内镜前端的超声探头有5MHz和7MHz两个频率可方便选择，完备的多谱勒及彩色血流功能，清晰显示血管及血流，是减少并发症及准确定位的关键。注射选用EUSN-20-CPN(Wilson-Cook产品)专用注射针，该针型前端有多个侧孔，注射后药物弥散效果好。药品用无菌消毒的95%～98%无水酒精。

3. 方法：进入胃内后，利用EUS的胃镜视野大致观察胃壁情况，了解有无局部静脉曲张，有无十二指肠梗阻。开启超声系统、水囊注水，对准胃肠壁探查，显示胰腺占位，寻找离胃肠壁最近距离，用多谱勒显示血流和血管声像图，确定预计要穿刺的路径上无明显血流回声，经内镜活检孔将穿刺针连同针芯准确刺入肿块内，拔除针芯、回抽无血，首先行组织细胞病理穿刺[3]，然后在肿瘤中央注入98%无水酒精10～30 ml，以酒精不超过肿瘤边界为度，尽量使用多个位点注射，以求最大效果。注射过程注射针始终处于超声的监控下。用文献[3]方法对3例疼痛分数超过6(VAS评分)癌痛者行CPN处理。

4. 术后处理：入选病例全部用健择处理，术后验血淀粉酶、白细胞，记录治疗后瘤体大小、腹痛改善情况。2例用健择6个疗程后肿瘤无缩小，改用酒精注射后瘤体明显缩小，1例健择疗程完成后发现肝及肺转移，用无水酒精注射原发病灶得到控制。

结 果

一、临床表现

8例黄疸、2例同时上腹痛，9例表现食欲减退，1例表现腹水。CA19-9最高大5 000 U/ml以上，2例始终CA19-9无升高，1例表现AFP升高，230 μg/L，见表1。

表1 临床表现

癌部位	消瘦	疼痛	CA19-9	CEA	胆红素
胰体尾	2	1	500.34 ± 897.36	25 ± 9.87	27 ± 2.98
胰头癌	8	1	890.56 ± 234.23	40 ± 8.90	298 ± 99.6

二、影像学表现

10 例均为晚期胰腺癌无法手术病例，9 例B 超、10 例CT 及超声内镜(EUS)均发现占位。EUS 病灶显示低回声，3 例回声略显不均，边界不清，1 例胰体尾交界部癌病变延至腹腔干、腹腔干血管无法显示。清晰显示血管侵犯的表现，3 例发现门静脉高回声壁消失，管腔变小；2 例腹腔干周围肿大淋巴结，大小分别为 15 mm × 7 mm、13 mm × 10 mm，表 2。

表 2 影像学占位表现

	实性病变	血管侵犯	淋巴结肿大	腹腔干侵犯	远处转移
B 超	9	4	4	0	1
CT	10	7	6	2	2
EUS	10	9	8	3	2

三、EUS 穿刺及病理改变

穿刺见组织细条为穿刺结束标准或当场细胞涂片见肿瘤细胞则视为穿刺成功。用 10 ml 空针抽成负压吸引，最多穿刺达4 次获标本者。胰头部穿刺次数略多于体尾部穿刺。EUS-FNA 9 例获满意抽吸标本，1 例取材不满意(图 1、图 2)。

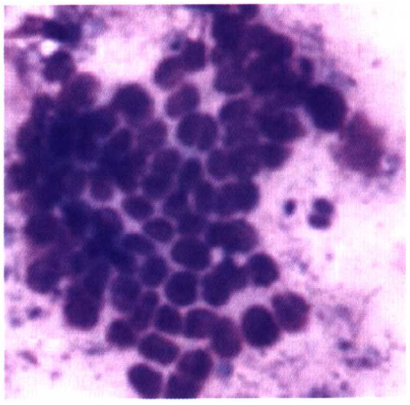

图 1 EUS 引导穿刺细胞学

四、疗效

平均注射 4 次，平均注射量 15 ml，10 例瘤体均不同程度缩小，4 例 7 个月时仍缩小超过 50%，3 例缩小 70%以上，缩小 70%的病例中 1 例注射前显示门静脉因受侵犯几乎完全闭合，注射第 3 次时门静脉显示出来，到第 4 次注射已可清楚显示门静脉管壁；1 例因肝转移、肺转移，注射两次后肿瘤缩小，但用化疗反应较大停止注射治疗。1 例注射前已表现十二指肠梗阻，经 2 次注射后梗阻症状消失。3 例合并腹痛，疼痛分数 6 分以上，在对胰腺癌瘤体注射的同时行无水酒精腹腔干阻滞治疗，疼痛分数明显下降，随着瘤体多次注射后疼痛几乎消失。8/10 例注射后次日，乏力明显减轻，食欲增加(图 3～8)。胰头癌者术前行 ERCP 置入胆管塑料支架已无黄疸，全部病例术后无胆红素升高。

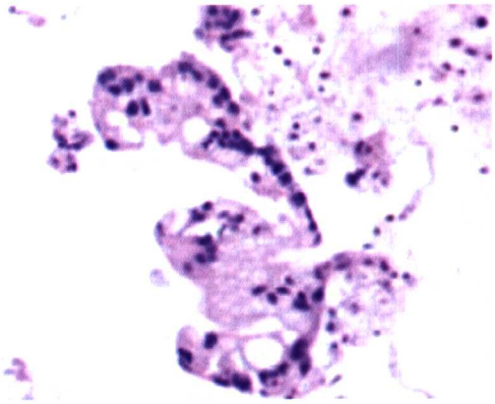

图 2 EUS 引导穿刺组织病理

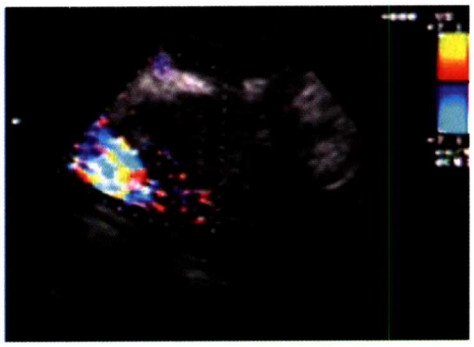

图 3 EUS 胰腺占位、门静脉受侵犯

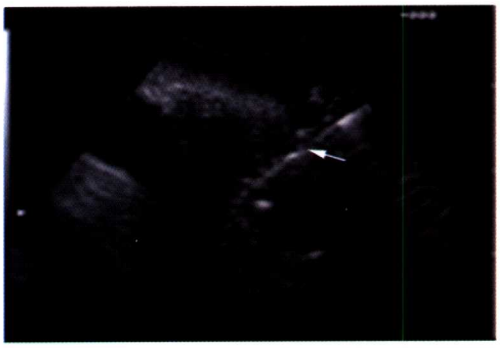

图 4 EUS-FNA 取病理，箭头示穿刺针

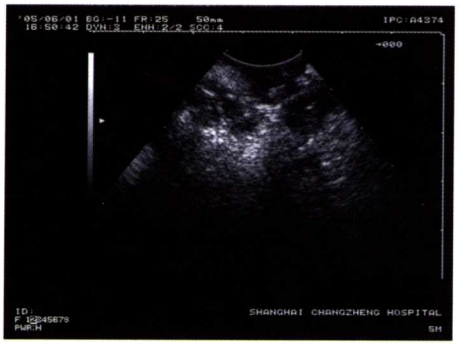

图5 EUS引导下无水酒精注射，注射区域呈云雾状高回声

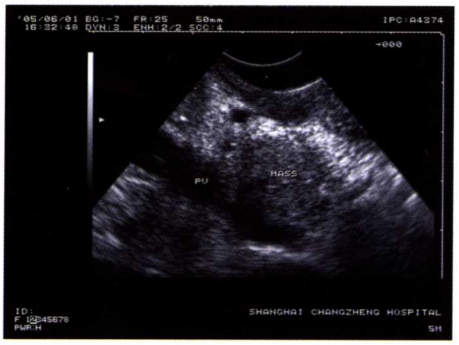

图6 无水酒精注射后，肿瘤缩小，门静脉显现

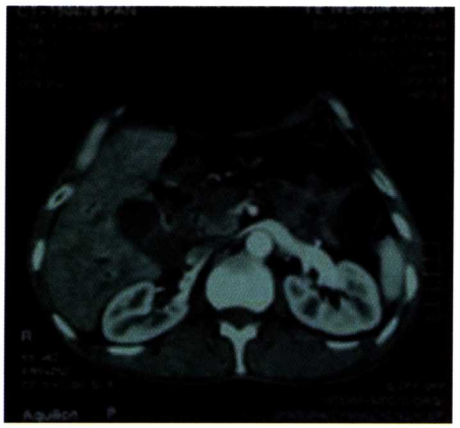

图7 无水酒精治疗前

五、并发症

无发热、出血，无穿孔发生。咽痛5例、上腹不适7例，3 d内食欲减退6例，轻度腹痛3例，2例出现较剧烈腹痛2 h，全部病例无胰腺炎发生及黄疸加重病例。腹腔神经丛组织者1例体位性低血压，6 h缓解，1例短暂腹泻不到48 h用止泻药治愈。血淀粉酶及白细胞观察：血淀粉酶术后保持在200U/L以内，白细胞升高2 d者5例，表4。

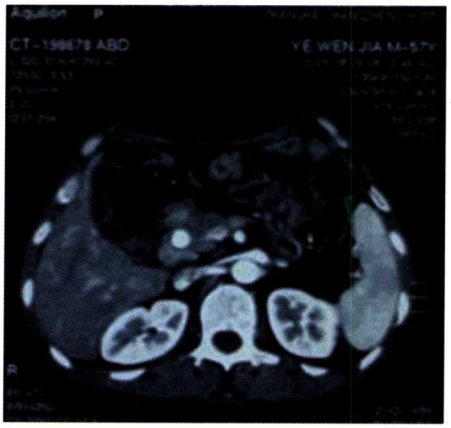

图8 无水酒精治疗后（发病后9个月）

表4 注射后血淀粉酶、白细胞

时间(h)	血淀粉酶(U/ml)	白细胞($\times 10^9$/L)
3	58.24 ± 6.78	7.56 ± 2.24
6	104.72 ± 51.32	10.26 ± 3.65
24	76.21 ± 9.62	8.35 ± 1.67

讨 论

胰腺癌发病呈上升趋势，无有效治疗方法，为最难治疗的癌肿之一。本文在不开腹情况下经EUS引导用无水酒精直接注射癌体，10例瘤体均有缩小，3/10例肿瘤缩小70%以上，4/10例缩小50%以上，且存活超过7月以上无明显不适。在治疗瘤体的同时尚可对癌痛进行无水酒精神经溶解止痛。全部病例中无胰腺炎及其他严重并发症发生，可望成为综合治疗胰腺癌的重要部分。

胰腺癌的综合治疗包括放疗、热疗、化疗、生物治疗及中医中药处理，胰腺肿瘤对放疗和化疗均为疗效最差者。随着定位技术准确性的提高，放疗虽可达到20%疗效，但因胰腺癌特殊位置使其并发症高居不下，且其价格昂贵，难以广泛应用。近年来最有希望的健择联合或单用治疗胰腺癌，对改善生活质量和短暂提高生存期确实有作用，但因其作用机制决定健择不可能消除或使肿瘤明显缩小[4]。介入治疗尤其是疗效卓著的栓塞介入治疗在胰腺癌被视为禁区，胰腺血管分布不像肝脏那样是双套血供，血管本身也不及肝脏丰富，血管网比较少，一旦血管栓塞或破坏胰腺炎并发症发生率很高，且容易合并重症胰腺炎。多种实体瘤可用无水酒精注射消减肿瘤体

积。原发性肝癌酒精介入4年生存率达50%，尤其＜3 cm肝癌，4年生存率超过60%[5]。酒精可导致细胞脱水、凝固坏死，可直接消减瘤细胞。截止目前，胰腺癌酒精注射未见类似的治疗和报道。本文10例胰腺癌经EUS引导无水酒精注射肿瘤体积都有缩小，即便是发生明显转移者，最大缩小达70%以上，且治疗后症状可获得改善，疗效肯定。有可能者选用无全身广泛转移的病例，疗效可能更显著。

超声内镜是目前显示胰腺癌及引导介入治疗最理想的手段，借助其前端的高频探头，可敏感显示病变，对肿瘤边界勾画十分清晰，彩色多谱勒血流图可成像细小的血管。经EUS引导注射，既可观察针道，又可显示注射药物分布情况，也能避免血管损伤，作为介入引导工具对胰腺癌治疗有着重要临床意义[6]。我们对病例除了影像学确诊外，均进行了病理学检查，9例获细胞病理诊断，治疗针对性十分明确。我们体会，每例注射2～3针，注射总量不超过30 ml、注射范围不超过肿瘤边界、清楚显示及避开血管等比较安全，也保证了EUS介入治疗特异性发挥。

胰腺癌酒精注射结合健择化疗可能疗效更加明显。实体肿瘤化疗不可能将肿瘤细胞全部一次性杀灭干净，即便大剂量化疗法。肿瘤中央往往是化疗药物最难达到的部位，对化疗最不敏感，容易形成耐药、容易有肿瘤细胞的冬眠。所以我们在对肿瘤中心位置注射酒精后，接着用健择化疗，不仅提高药物疗效，也可减少耐药的形成。剩下周边的癌细胞数量减少，血供相对丰富，也是化疗比较敏感的区域。

有效治疗的基础上不发生或极少发生胰腺炎是本方法成功的关键。本文10例胰腺癌者全部无胰腺炎发生，全部无胆管炎及黄疸加重。可能与术前均置入胆管支架、常规预防胰腺炎有关。70%以上的胰腺癌为胰腺导管组织起源，所以癌组织中的导管受到了阻塞，酒精沿导管扩散的机会不大，超声内镜可清楚显示注射针道和肿瘤边界[7]，酒精注入正常胰腺的可能性大为减小，准确性得以保证，加之有效的预防，胰腺炎发生率几乎不可能。事先置入胆管支架，提高了胆道的显示率，即便是酒精直接注入胆道，由于支架的支撑作用，其并发症也大大减轻。

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奥林巴斯EVIS LUCERA电子内镜系统的临床应用

奥林巴斯EVIS LUCERA(即260系列)的诞生,不仅仅是对原有内镜产品的升级换代,而且是对消化道内镜诊疗技术产生深远影响的技术革新。这一创新主要着眼于两个方面:更精确的诊断性能与提供更便捷的内镜检查与治疗。

奥林巴斯将HDTV高清晰度成像系统运用到EVIS LUCERA中,图像由此变得更加细腻、鲜明。临床上特别对微血管及细微的黏膜结构变化的再现更为有效,为发现早期病变提供了可靠的依据。从逼真的高清晰度HDTV图像到精密的图像分析手段,如IHb色彩强调、IHb模拟色图;从新一代的图像构造强调到电子放大功能,EVIS LUCERA不仅支持目前的内镜诊疗技术,而且还充分挖掘内镜诊疗的巨大潜能。

奥林巴斯EVIS LUCERA在操作上,也变得更加简便与迅速。适合儿童与狭窄患者的超细胃镜、软硬度可变并且具有放大功能的肠镜、提高ERCP附件快速交换的新颖十二指肠镜、图像冻结的快速与实时、内镜信息的记忆功能等,都给我们操作带来了实实在在的便利。

适应型的IHb色彩强调与IHb模拟色图在消化道临床的应用

早期发现,早期治疗,有利于提高疾病的治愈率。在内镜诊断中,早期的病变一般难以察觉,提高内镜诊断能力是我们发现早期病变的前提。目前内镜提高病变诊断率的方法主要有二类,一类是通过黏膜的构造(凹凸)进行强调,另一类是通过黏膜的色调变化进行强调。黏膜的构造强调技术已经在普通的电子内镜上被广泛采用,而色调变化的强调技术则目前尚未在内镜中普及。

适应型IHb色彩强调功能,能进一步提高病变的诊断性能。消化道黏膜的色调,基本上是由血红蛋白这一天然色素形成,测定血红蛋白的浓度即能测定消化道黏膜的色调。EVIS LUCERA通过电子内镜图像处理数据对血红蛋白浓度进行定量测定与分析,使图像中黏膜的红、白色调对比度增强,突出病变范围,提高对病变的诊断率。

一、IHb色彩强调的原理

IHb是英文Index of Hemoglobin的缩写,即血色素指数。IHb色彩强调功能的原理,简单地讲就是顺次式扫描的内镜系统中,主机通过先端部CCD(黑白CCD)获得的R、G、B讯号中每一像素所接受颜色(R与G)信号的强弱,根据指数公式IHb = 32log2(Vr/Vg)进行演算,推算出血红素浓度的指数,通过将高于观察图像IHb平均值的像素进一步向红色强调,将低于平均值的像素进一步向白色强调,使得黏膜内容易忽略的细微色调变化得以强调,清晰地显示出发红或褪色的色调变化,由此把隐藏的病变突显出来,提高诊断率。

以图1为例,我们把指数值分成三个区域,有平均值以上的发红部分,也有平均值以下的褪色部分。

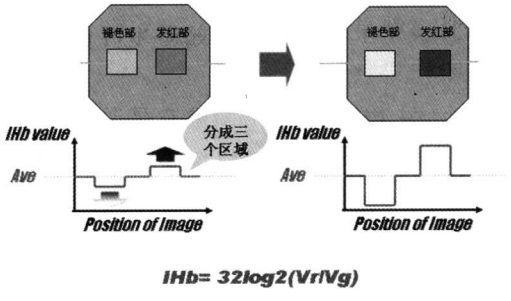


图1 IHb色彩强调功能的原理图

二、适应型IHb色彩强调在上消化道诊断中的应用

胃黏膜血管丰富，其色调一直是胃镜诊断的重要指标。通常来说，黏膜的发炎、溃疡以及肿瘤等病变的部位，因血管增生或黏膜损伤等原因，该部位血管比较充沛，也就是讲血红色素浓度较高，红色比较明显。因此我们可以通过IHb色彩强调容易地对病变的存在做出判断。

下面我们来举例说明适应型IHb色彩强调的临床应用。

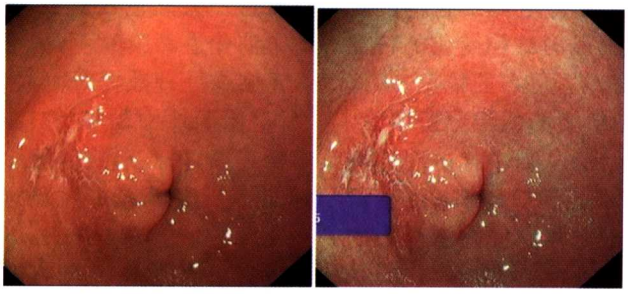
1.对早期胃癌的诊断价值

(1)使病变和背景黏膜的色差变大。

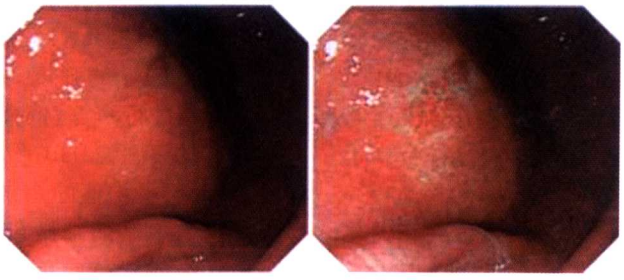
(2)通过使背景黏膜的图样、血管图像更清晰，相对容易识别病变。

(3)放大观察时可使微血管构造图像，特别是微小血管变密的部分更加清晰。

(4)有助于判断胃癌分化程度，高分化型胃癌一般为发红色调，而低分化型胃癌则为褪色色调。



高分化型胃癌色彩强调前　　高分化型胃癌色彩强调后



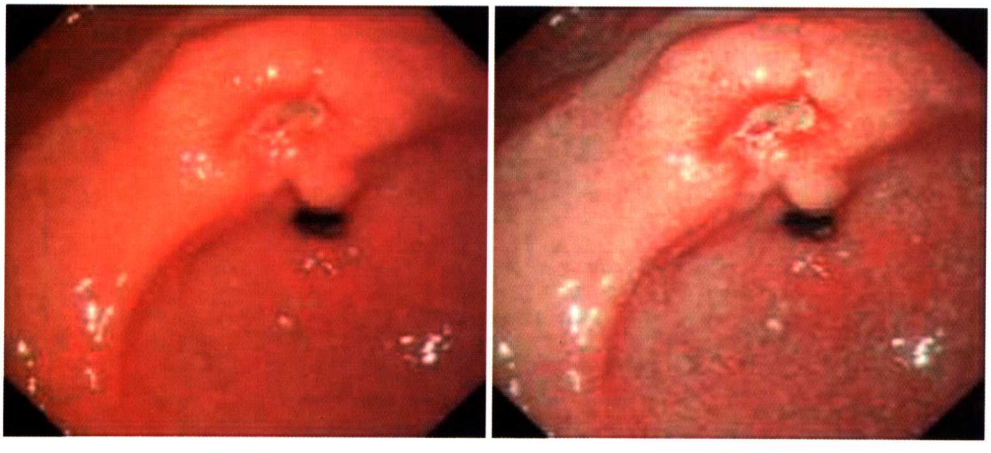
未分化型胃癌色彩强调前　　未分化型胃癌色彩强调后

2.对消化性溃疡的诊断价值

(1)能更清晰地显示溃疡边界。

(2)有助于溃疡的分期。

(3)有助于判断溃疡是否完全愈合。

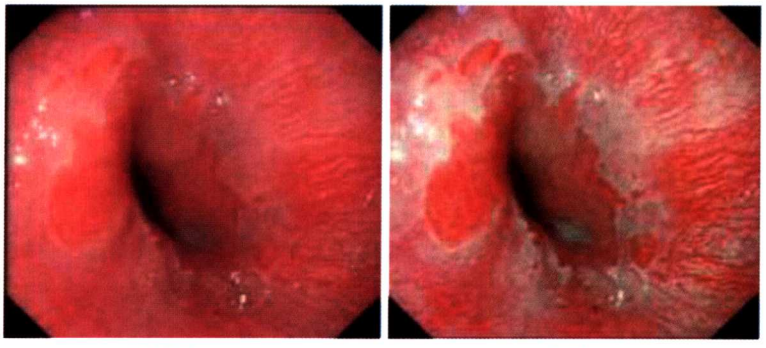


色彩强调前　　色彩强调后

3. 对Barrett 食管的诊断价值

(1)更清晰地显示鳞状上皮与柱状上皮的交界。

(2)Barrett 上皮合并不典型增生常表现为褪色性病灶。



色彩强调前　　色彩强调后

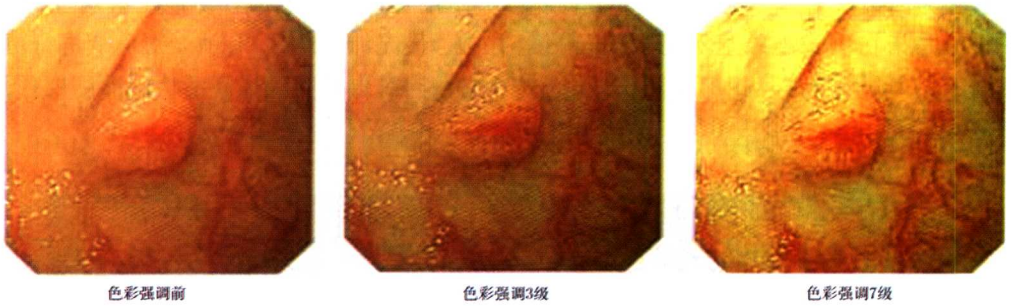
三、适应型IHb 色彩强调处理在下消化道诊断中的应用

结肠疾病的内镜诊断，无论是肿瘤性或炎性疾病，均应从病变部位黏膜的色调以及形态、周围血管网的变化等着手。因此通过适应型IHb 色彩强调处理，可以提高肠道疾病的诊断率。

1. 对结肠肿瘤的诊断价值

在结肠肿瘤的内镜诊断中，隆起型的病变，普通内镜也可以做出判断，但对于表面型肿瘤，有时根据肉眼观察难以诊断其存在性。内镜下可以通过肿瘤部位的发红、容易出血、肿瘤周围血管网的中断和皱褶的集中等发现表面型肿瘤。而通过色彩强调，则容易识别肿瘤部位的发红和周围血管网的变化，从而可以提高表面型肿瘤的诊断能力。

如图2 所示，普通内镜可见Ⅱa + Ⅱc 样的肿瘤，中央部的凹陷呈淡淡的发红。通过色彩的强调，凹陷部的发红更加清晰，容易判断病变的性质。病理组织学检查确认为高分化型黏膜内癌。



色彩强调前　　色彩强调3级　　色彩强调7级

图2　横结肠黏膜内癌

2. 对炎性结肠疾病的临床价值

在对炎性结肠疾病的内镜诊断中，色彩强调对于再生血管的炎症治愈过程的评价、跟踪观察溃疡性结肠炎(UC)长期病程 dysplasia 和 colitic cancer 的诊断等是有帮助的。

(1)对炎症治愈过程的评价

伴随UC 等炎性结肠疾病所发生的溃疡，在进入治愈过程后溃疡边缘部会增生再生血管，这种变化可以通过并用色彩强调的放大内镜清晰地进行观察。另外，当UC 进入到缓解期时，黏膜的血管可见度也开始恢复，使用色彩强调可以清晰观察血管的再生，用来进行病程的诊断。如图3 所示：结肠镜观察可见横结肠有大块的不规则溃疡，周围黏膜发红并呈浮肿状(A、B)，色彩强调后的放大内镜观察，可

清晰地观察到在溃疡边缘(C)有再生血管增生，可知溃疡正处于愈合过程之中。

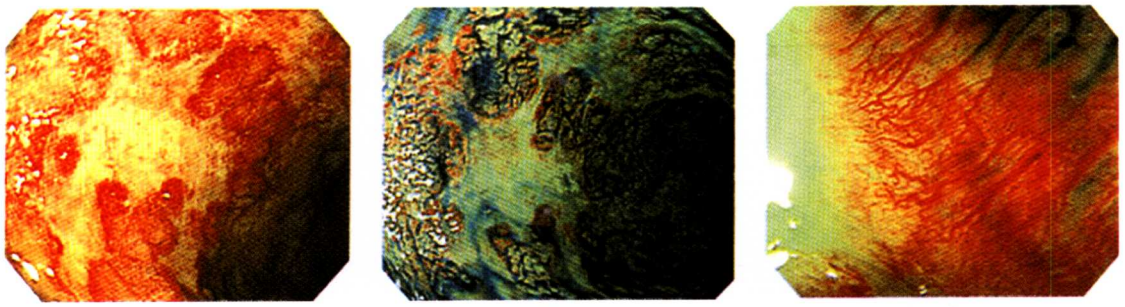


图3 溃疡性结肠炎

(2)对溃疡性结肠炎(UC)长期病程的跟踪观察

对于UC的长期病程，dysplasia和colitic cancer的危险性很高，必须利用肠镜定期进行复查。在进行dysplasia诊断时，必须着眼于不规则的黏膜隆起以及黏膜表面的发红和血管网的变化等内镜观察。色彩强调后可使黏膜表面的色调变化更加清晰，可以用来进行dysplasia和colitic cancer的诊断。但是，如果存在活动性炎症时，需要注意炎症所附带的发红也会被强调，增加了假阳性病变的可能性。

四、IHb模拟色图和IHb平均值的临床应用

奥林巴斯EVIS LUCERA在IHb色彩强调的基础上，增加了“IHb模拟色图显示”和“IHb平均值显示”两项功能，它为内镜诊断提供更多研究的方向。

IHb平均值是指在图像冻结显示时，根据内镜图像求出各像素的IHb值，将其相加计算出IHb平均值而显示出来。现在日本的一些专家正在研究将该IHb平均值应用于幽门螺杆菌的除菌判断中。通过一组患者的数据证明，当分界值设定为60时，*Hp*感染的敏感性为91%，特异性为95.2%，诊断正确性为98.7%。并且患者感染了*Hp*，IHb平均值会趋于高值，而当除菌治疗成功后，IHb平均值则趋向于低值。因此IHb平均值的显示，在评估病情变化与治疗效果上给我们提供了更多有价值的参考数据。

IHb色图是指在图像冻结时，主机在IHb色彩强调的基础上，通过计算出CCD中每一像素所接受到的IHb值的大小，再根据设定的色谱为每一像素编入其显示颜色，将颜色分为冷暖色调。IHb色图显示的作用就是更加容易地了解病变部位中血色(充血)最充沛的地方，有助于对病变进行更加详细的分析，同时也有利于提高活检的准确度(图4)。

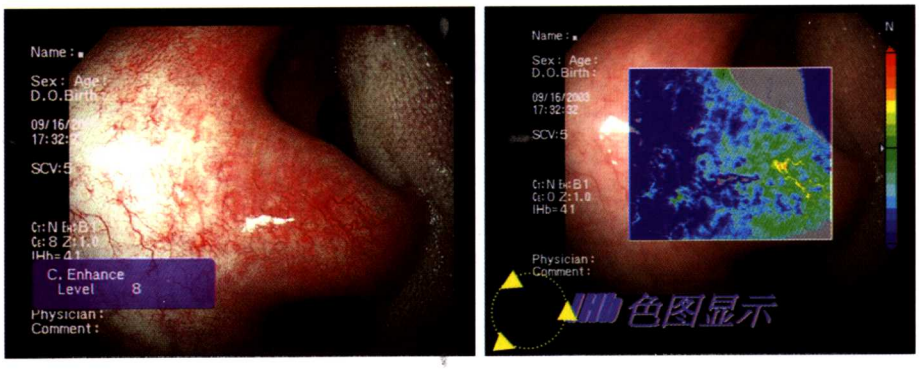


图4 IHb模拟色图和IHb平均值的显示

放大电子结肠镜的临床应用

奥林巴斯EVIS LUCERA的放大肠镜CF-H260AZI，因在其先端部内侧安装精密的微型驱动器装置，从而达到电动变焦的目的。操作时我们只要